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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

014122

OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: April 6, 2000

SUBJECT: ***Chlorpyrifos-Methyl:*** Evaluation of a 90-Day Subchronic Dietary Toxicity and Recovery Study in the Rat Submitted in Support of Reregistration of the Pesticide, Chlorpyrifos-Methyl.

PC Code: 059102
DP Barcode: D259305
Submission No.: S567933

FROM: Michelle Centra, Pharmacologist
Reregistration Branch III
Health Effects Division (7509C)

THRU: Steve Knizner, Branch Senior Scientist
Reregistration Branch III
Health Effects Division (7509C)

TO: Stephanie Nguyen, Chemical Review Manager
Kathy Monk, Branch Chief
Reregistration Branch II
Special Review and Reregistration Division (7508W)
and
Gary Bangs, Industrial Hygienist/Risk Assessor
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Health Effects Division (7509C)
and
John Doherty, Toxicologist
Reregistration Branch III
Health Effects Division (7509C)

Introduction:
Dow AgroSciences has requested that the Agency review the toxicology study entitled "Chlorpyrifos-Methyl (Reldan): Rat Subchronic Dietary Toxicity and Recovery Study" (MRID 44906902, 45048301) in support of reregistration of the pesticide, chlorpyrifos-methyl.

Action Requested:
Review the 90-day subchronic dietary toxicity study in the rat submitted to the Agency by Dow AgroSciences in support of reregistration of the pesticide, chlorpyrifos-methyl.

Agency's Response:
Reregistration Branch III has reviewed the 90-day subchronic feeding study in the rat (MRID 44906902, 45048301). Although the study was classified acceptable for regulatory purposes because it satisfies the guideline requirements for a subchronic toxicity study (OPPTS 870.3100, §82-1a) in the rat, this study was not submitted by the Registrant with the intent to fulfill any data gap(s) identified in the existing chlorpyrifos-methyl toxicology data base.

Given that the no observable adverse effect level (NOAEL) identified in both the 90-day subchronic toxicity study (MRID 44906902, 45048301) in rats and the 2-year combined chronic toxicity/carcinogenicity study (MRID 42269001) in rats is the same (NOAEL = 0.1 mg/kg/day) and that the later study was used to establish the RfD and the intermediate- and long-term exposure endpoints, this 90-day subchronic toxicity study will have no impact on the chlorpyrifos-methyl risk assessment.

Conclusion:
This study submitted by Dow AgroSciences is classified acceptable and it satisfies the guideline requirements for a subchronic toxicity study (82-1a) in rats. Although the study is acceptable for regulatory purposes, it will have no impact on the chlorpyrifos-methyl risk assessment.

The executive summary and study citations for this 90-day subchronic toxicity study are included in this memorandum and the data evaluation record (DER) is attached.

Study: Subchronic Oral Toxicity [Feeding] - Rat, Guideline OPPTS 870.3100 (§82-1a); MRID 44906902, 45048301

Study Citation(s): Barna-Lloyd, T., et al. (1990): Chlorpyrifos-Methyl (Reldan): Rat Subchronic Dietary Toxicity and Recovery Study; Health & Environmental Sciences-Texas, The Dow Chemical Company, Lake Jackson, Texas; Laboratory Study ID TXT:K-046193-026; Study Completion Date: June 18, 1990. (Unpublished) MRID NUMBER 44906902

Jachetta J.J. (2000): Additional Information for MRID 44906902-Chlorpyrifos-Methyl (Reldan): Rat Subchronic Dietary Toxicity and Recovery Study; Dow AgroSciences LLC, North American Research and Development, Indianapolis, Indiana; Study ID: JJJ02242000; Completion Date: February 24, 2000. (Unpublished) MRID NUMBER 45048301

Executive Summary: In a subchronic toxicity study (MRID 44906902, 45048301), primary groups of Fischer-344 rats (10/sex/dose) were administered Chlorpyrifos-methyl (Reldan F, 95.2% a.i.) in the diet at dose levels of 0 (control), 0.1, 1, 10 and 250 mg/kg/day for 13 weeks. Concurrent satellite groups, designated as recovery rats (10/sex/dose) were fed with either 0 or 250 mg/kg/day chlorpyrifos-methyl for 13 weeks and then allowed 4 weeks for recovery.

The clinical signs attributed to chlorpyrifos-methyl administration were urine staining, decreased condition of the haircoat and increased lacrimation. These effects occurred almost entirely in the 250 mg/kg/day chlorpyrifos-methyl dose group and were observed most often in female rats compared to male rats. Mean body weights of male rats dosed at 250 mg/kg/day were significantly decreased throughout the13 week treatment period (3.0-10.1%). Similarly, high-dose females had significantly lower body weights (5.0-14.1%). Mean body weight gains were also significantly decreased in males (17.2%) and in females (26.3%) during dosing (study days 0-91). High-dose females appeared to consume less food (3.3-13%) compared to control animals throughout the treatment period.

Measurements of plasma cholinesterase activity at 6 weeks showed significant dose-related decreases; enzyme inhibition was observed at 1 mg/kg/day (12.5% in males; 34.1% in females) and reached maximum levels in males (48.7%) and in females (87.3%) at 250 mg/kg/day. Following the 13 week dosing interval, plasma cholinesterase activity was also significantly decreased in a dose-dependent manner (males:7.3-53.7% at dose levels of 0.1 mg/kg/day and above; females: 33.8-86.6% at dose levels of 1 mg/kg/day and above). Significant decreases were observed in the 6 week red blood cell cholinesterase activity levels (25.5% at 10 mg/kg/day; 30.1% at 250 mg/kg/day) and in the 13 week brain cholinesterase activity levels (17.0% at 10 mg/kg/day; 62.6% at 250 mg/kg/day) of male rats. Similarly, the 6 week red blood cell cholinesterase activity level was significantly decreased at 10 mg/kg/day (31.5%) and at 250 mg/kg/day (25.4%), but remained significantly inhibited and relatively unchanged (27.7%) only at 13 weeks in the high-dose group female rats. Brain cholinesterase activity at 13 weeks was significantly inhibited in the 10 mg/kg/day (14.5%) and 250 mg/kg/day (58.9%) group female rats.

The absolute and relative group mean adrenal weights were significantly increased at 13 weeks in high-dose males (58.0% and 81.6%, respectively) and in high-dose females (64 and 90%, respectively). Adrenal weights, both absolute and relative, were also significantly increased at 10 mg/kg/day in females (40.0% and 45.8%, respectively); only absolute adrenal gland weights were significantly increased in males (10.0%). Absolute and relative liver weights were increased in high-dose males (22.5% and 39.0%, respectively) and in high-dose females (20.5% and 40.2%, respectively).

In all rats fed chlorpyrifos-methyl at dose levels of 10 and 250 mg/kg/day, microscopic lesions identified in the adrenal glands consisted of hypertrophy and or vacuolation of the cells of the zona fasciculata. Also noted within the zona fasciculata of mid- and high-dose female rats were scattered necrotic foci, suggesting that the pathologic process within the adrenal glands may have

progressed to necrosis. Microscopic examination of the liver revealed hypertrophy of centrilobular hepatocytes that was observed only in male rats at 250 mg/kg/day.

By the end of the recovery period (study week 17), most animals showed either a partial or complete reversal of the adverse effects observed during the 13 week dosing period. These effects included clinical signs, body weight, body weight gain, food consumption, cholinesterase activity (plasma, red blood cell and brain), hematology parameters, absolute and relative organ weights, hypertrophy, vacuolation and necrosis of the adrenal glands and hypertrophy of centrilobular hepatocytes.

For females, the LOAEL for plasma cholinesterase inhibition was 1.0 mg/kg/day and the NOAEL was 0.1 mg/kg/day . For males, the LOAEL for plasma cholinesterase inhibition was 0.1 mg/kg/day and the NOAEL was not established.

The LOAEL for red blood cell and brain cholinesterase inhibition was 10 mg/kg/day and the NOAEL was 1 mg/kg/day.

The LOAEL for systemic toxicity was 10 mg/kg/day based upon histopathology (hypertrophy, vacuolation and necrosis) of the adrenal glands and the NOAEL was 1 mg/kg/day.

This study is classified **Acceptable-Guideline.** It is recognized that for three of the thirteen weeks of dosing, the control diet contained 0.251 ppm (0.0126 mg/kg/day) chlorpyrifos-methyl (approximately 12.6% of the low dose). However, this small contaminating quantity was not considered sufficient to compromise the interpretation of the data. Therefore, this study does meet the guideline requirements for a subchronic toxicity study (82-1a) in rats.

cc: John Doherty (RRB III), Michelle Centra (RRB III), Gary Bangs (RRB III), Steve Knizner (RRB III), Stephanie Nguyen (SRRD, RRB II), Kathy Monk (SRRD, RRB II).

enclosure —

014122

CHLORPYRIFOS-METHYL (RELDAN F) **SUBCHRONIC TOXICITY (82-1a)**

EPA Primary Reviewer: Michelle M. Centra
Reregistration Branch III (7509C)

Michelle M. Centra 4/18/00
(Signature and Date)

EPA Secondary Reviewer: John Doherty
Reregistration Branch III (7509C)

4/18/00
(Signature and Date)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [feeding] - Rat
Species: Rat Guideline: OPPTS 870.3100 [§82-1a]

EPA ID NUMBERS: DP Barcode: D259305 PRAT Case No.: 292273
PC Code: 059102 Submission No.: S567933

TEST COMPOUND (PURITY): Chlorpyrifos-methyl (95.2% a.i.)

SYNONYM(S)/COMPOSITION: Reldan F; $C_7H_7Cl_3NO_3PS$

CITATION(S):

Barna-Lloyd, T., et al. (1990): Chlorpyrifos-Methyl (Reldan): Rat Subchronic Dietary Toxicity and Recovery Study; Health & Environmental Sciences-Texas, The Dow Chemical Company, Lake Jackson, Texas; Laboratory Study ID TXT:K-046193-026; Study Completion Date: June 18, 1990. (Unpublished) MRID NUMBER 44906902

Jachetta J.J. (2000): Additional Information for MRID 44906902-Chlorpyrifos-Methyl (Reldan): Rat Subchronic Dietary Toxicity and Recovery Study; Dow AgroSciences LLC, North American Research and Development, Indianapolis, Indiana; Study ID: JJJ02242000; Completion Date: February 24, 2000. (Unpublished) MRID NUMBER 45048301

SPONSOR: Dow Agrochemical, Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 44906902, 45048301), primary groups of Fischer-344 rats (10/sex/dose) were administered Chlorpyrifos-methyl (Reldan F, 95.2% a.i.) in the diet at dose levels of 0 (control), 0.1, 1, 10 and 250 mg/kg/day for 13 weeks. Concurrent satellite groups, designated as recovery rats (10/sex/dose) were fed with either 0 or 250 mg/kg/day chlorpyrifos-methyl for 13 weeks and then allowed 4 weeks for recovery.

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250 mg/kg/day chlorpyrifos-methyl dose group and were observed most often in female rats compared to male rats. Mean body weights of male rats dosed at 250 mg/kg/day were significantly decreased throughout the13 week treatment period (3.0-10.1%). Similarly, high-dose females had significantly lower body weights (5.0-14.1%). Mean body weight gains were also significantly decreased in males (17.2%) and in females (26.3%) during dosing (study days 0-91). High-dose females appeared to consume less food (3.3-13%) compared to control animals throughout the treatment period.

Measurements of plasma cholinesterase activity at 6 weeks showed significant dose-related decreases; enzyme inhibition was observed at 1 mg/kg/day (12.5% in males; 34.1% in females) and reached maximum levels in males (48.7%) and in females (87.3%) at 250 mg/kg/day. Following the 13 week dosing interval, plasma cholinesterase activity was also significantly decreased in a dose-dependent manner (males:7.3-53.7% at dose levels of 0.1 mg/kg/day and above; females: 33.8-86.6% at dose levels of 1 mg/kg/day and above). Significant decreases were observed in the 6 week red blood cell cholinesterase activity levels (25.5% at 10 mg/kg/day; 30.1% at 250 mg/kg/day) and in the 13 week brain cholinesterase activity levels (17.0% at 10 mg/kg/day; 62.6% at 250 mg/kg/day) of male rats. Similarly, the 6 week red blood cell cholinesterase activity level was significantly decreased at 10 mg/kg/day (31.5%) and at 250 mg/kg/day (25.4%), but remained significantly inhibited and relatively unchanged (27.7%) only at 13 weeks in the high-dose group female rats. Brain cholinesterase activity at 13 weeks was significantly inhibited in the 10 mg/kg/day (14.5%) and 250 mg/kg/day (58.9%) group female rats.

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complete reversal of the adverse effects observed during the 13 week dosing period. These effects included clinical signs, body weight, body weight gain, food consumption, cholinesterase activity (plasma, red blood cell and brain), hematology parameters, absolute and relative organ weights, hypertrophy, vacuolation and necrosis of the adrenal glands and hypertrophy of centrilobular hepatocytes.

For females, the LOAEL for plasma cholinesterase inhibition was 1.0 mg/kg/day and the NOAEL was 0.1 mg/kg/day . For males, the LOAEL for plasma cholinesterase inhibition was 0.1 mg/kg/day and the NOAEL was not established.

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This study is classified **Acceptable-Guideline.** It is recognized that for three of the thirteen weeks of dosing, the control diet contained 0.251 ppm (0.0126 mg/kg/day) chlorpyrifos-methyl (approximately 12.6% of the low dose). However, this small contaminating quantity was not considered sufficient to compromise the interpretation of the data. Therefore, this study does meet the guideline requirements for a subchronic toxicity study (82-1a) in rats.

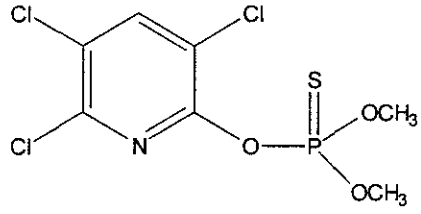
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Compound: Chlorpyrifos-methyl (Reldan F)

Description: granular crystalline solid
Lot/Batch number: 01214
Purity: 95.2 ± 0.3% a.i. as per Dow AgroSciences Certificate of Analysis dated November 13, 1990 (purity reported under separate MRID 45048301)
CAS Registry Number: 5598-13-0
Receipt date: July 15, 1996
Stability: The test compound was stable at room temperature.
Structure:



Other provided information: The test compound was stored at room temperature. Homogeneity of the test compound was verified at the 10 mg/kg/day concentration on the day of the first preparation. The actual concentrations of the test compound in the vehicle were verified for the 10 mg/kg/day concentration (the test compound was prepared a total of thirteen times during the study).

2. Vehicle(s):

The test material was mixed with feed (Purina Certified Rodent Chow #5002).

3. Test Animals:

Species: Rat
Strain: Fischer-344
Source: Charles River Breeding Laboratories
Age of animal: 4 weeks at arrival; 6-7 weeks old just prior to initiation of the study.
Body weight of animal: 117-136 g for males and 90-103 g for females just prior to the first day of treatment (study day -1).
Acclimation period: 14 days

Diet: Purina Certified Rodent Chow #5002 (Ralston Purina Co., St. Louis, MO , *ad libitum* (except during fasting and prior to sacrifice)
Housing: Individually housed in suspended stainless steel wire mesh cages.
Water: Tap water, *ad libitum*
Environmental conditions:
Temperature: 72°F
Humidity: Not reported
Air changes: 13 air changes/hour
Photoperiod: 12 hours light/dark

B. STUDY DESIGN AND METHODS:

1. In life dates: start: November 4, 1987 end: February 3, 1988

2. Animal assignment and treatment:

Each rat (70 males, 70 females) received an identifying metal ear tag showing a unique sequential number assigned from a computerized code list. Prior to the administration of the test material (study day -8), animals were stratified by body weight according to sex and assigned to test groups using a computerized procedure based on random numbers. The rats were further divided into two categories: primary groups and recovery groups (Table 1). For 13 weeks, the primary group rats (10/sex/dose) were administered chlorpyrifos-methyl (95.2% a.i.) in the diet at dose levels of 0, 0.1, 1.0, 10.0 or 250.0 mg/kg/day. Concurrently, the recovery group rats (10/sex/dose) were fed either 0 or 250 mg/kg/day chlorpyrifos-methyl in their diets. Following the 13 week treatment period, the primary group animals were necropsied whereas the recovery group animals were given untreated diets for 4 weeks and then necropsied.

Table 1. Study Design and Animal Assignment

Dose (mg/kg/day)	Primary Group Number of Rats		Recovery Group Number of Rats		Total Number of Rats/Dose
	Males	Females	Males	Females	
0	10	10	10	10	40
0.1	10	10	---	---	20
1.0	10	10	---	---	20
10.0	10	10	---	---	20
250.0	10	10	10	10	40
Total Number of Rats	100		40		140

3. Diet preparation and analysis

Chlopyrifos-methyl test diets were prepared weekly by serial dilution from a 1% or 2% premix in commercial stainless steel mixing equipment. The test diets were prepared to contain sufficient chlorpyrifos-methyl based on the weekly body weights and food consumption to achieve doses of 0, 0.1, 1.0, 10 and 250 mg/kg/day.

Results-

Homogeneity Analysis: Homogeneity of the test diet to be fed to the 10 mg/kg/day dose group was measured prior to the start of treatment (study day -13). This diet preparation was shown to be adequate based on test samples taken from the center top, center bottom, side top or side bottom of the mixing apparatus.

Table 2a. Homogeneity Analysis

Sample Category	Targeted Concentration (ppm)	Measured Concentration (mean ± S.D., ppm)	Percent (%) of Targeted Concentration[a]
Center, Top	167	185 ± 2.0	110.8
Center, Bottom	167	173 ± 4.0	106.1
Side, Top	167	160 ± 4.0	95.8
Side, Bottom	167	171 ± 21.0	102.4

[a]The percent (%) of chlorpyrifos-methyl target concentrations were calculated by the reviewer using data provided in Table 2, page 35 of the study report.

Stability Analysis: The test diets prepared prior to dosing (study day -13) and on study days 7, 14 and 28 were analyzed for stability following 14 day storage at room temperature. Measured concentrations of chlorpyrifos-methyl for the 10 mg/kg/day dietary level were decreased by 5.4% or less compared to the targeted concentration for up to14 days. Since these differences were within the precision of the analytical procedure, the stability of the test material in prepared diets was shown to be adequate.

Table 2b. Stability Analysis			
Sample Category	Targeted Concentration (ppm)	Measured Concentration (ppm, mean ± S.D.)	Percent (%) of Targeted Concentration
Day 0	167	172 ± 10.0	103.0
Day 7	167	158 ± 6.0	94.6
Day 14	167	164 ± 18.0	98.2
Day 28	167	144 ± 3.0	86.2

The percent (%) of chlorpyrifos-methyl target concentrations were calculated by the reviewer using data provided in Table 2, page 35 of the study report.

Concentration Analysis: The concentrations of chlorpyrifos-methyl measured in test diets 1, 2 and 3 which were prepared on study days 1, 50 and 85, respectively, were decreased by 12.1% or less. All measured test material concentrations were comparable to the targeted concentrations and were within acceptable limits.

Table 2c. Concentration Analysis			
Sample Category	Diet 1	Diet 2	Diet 3
Targeted Concentration (ppm)			
0 ppm, Control	0	0	0
0.1 ppm, Males Females	1.20 1.08	1.62 1.28	1.94 1.57
1 ppm, Males Females	$(1.20) \times 10^1$ $(1.06) \times 10^1$	$(1.65) \times 10^1$ $(1.25) \times 10^1$	$(1.89) \times 10^1$ $(1.52) \times 10^1$
10 ppm, Males Females	$(1.19) \times 10^2$ $(1.07) \times 10^2$	$(1.62) \times 10^2$ $(1.31) \times 10^2$	$(1.95) \times 10^2$ $(1.54) \times 10^2$
50 ppm, Males Females	$(5.96) \times 10^2$ $(5.32) \times 10^2$	$(8.28) \times 10^2$ $(6.12) \times 10^2$	$(9.64) \times 10^2$ $(7.39) \times 10^2$
250 ppm, Males Females	$(3.01) \times 10^3$ $(2.60) \times 10^3$	$(3.92) \times 10^3$ $(3.10) \times 10^3$	$(4.41) \times 10^3$ $(3.65) \times 10^3$
Premix (1% or 2%)	$(1.00) \times 10^4$	$(1.00) \times 10^4$	$(2.00) \times 10^4$
Measured Concentration (ppm, mean ± S.D.)			
0 ppm, Control	None Detected[a]	0.25 ± 0.01[b]	None Detected[c]
0.1 ppm, Males Females	1.20 ± 0.04 1.33 ± 0.07	1.91 ± 0.25 1.84 ± 0.20	1.86 ± 0.07 1.38 ± 0.04

1.0 ppm, Males	$(1.17 \pm 0.01) \times 10^1$	$(1.55 \pm 0.03) \times 10^1$	$(1.75 \pm 0.11) \times 10^1$
Females	$(1.15 \pm 0.03) \times 10^1$	$(1.36 \pm 0.02) \times 10^1$	$(1.70 \pm 0.21) \times 10^1$
10 ppm, Males	$(1.23 \pm 0.03) \times 10^2$	$(1.51 \pm 0.11) \times 10^2$	$(1.88 \pm 0.08) \times 10^2$
Females	$(1.10 \pm 0.01) \times 10^2$	$(1.26 \pm 0.01) \times 10^2$	$(1.45 \pm 0.02) \times 10^2$
50 ppm, Males	$(5.88 \pm 0.05) \times 10^2$	$(7.64 \pm 0.10) \times 10^2$	$(9.63 \pm 0.14) \times 10^2$
Females	$(5.36 \pm 0.05) \times 10^2$	$(6.32 \pm 0.15) \times 10^2$	$(7.66 \pm 0.21) \times 10^2$
250 ppm, Males	$(2.92 \pm 0.01) \times 10^3$	$(3.58 \pm 0.03) \times 10^3$	$(4.29 \pm 0.03) \times 10^3$
Females	$(2.55 \pm 0.01) \times 10^3$	$(2.86 \pm 0.09) \times 10^3$	$(3.67 \pm 0.04) \times 10^3$
Premix (1% or 2%)	$(9.95 \pm 0.03) \times 10^3$	$(8.86 \pm 0.24) \times 10^3$	$(1.93 \pm 0.14) \times 10^4$
Percent (%) of Targeted Concentration			
0.1 ppm, Males	100.0	117.9	96.9
Females	123.1	143.8	87.9
1.0 ppm, Males	97.5	93.9	92.6
Females	108.5	108.8	111.8
10 ppm, Males	103.4	93.2	96.4
Females	102.8	96.2	94.2
50 ppm, Males	98.7	92.2	99.9
Females	100.8	103.3	103.7
250 ppm, Males	97.0	91.3	97.3
Females	98.1	92.3	100.5
Premix (1% or 2%)	99.5	88.6	96.5

[a]Limit of detection, 0.53 ppm.
[b]Limit of detection, 0.157 ppm.
[c]Limit of detection, 0.63 ppm.
The percent (%) of targeted chlorpyrifos-methyl concentration in prepared diets were calculated by the reviewer using data from Table 2, page 35-36 of the study report.

According to the study authors, analyses of test material concentration on study day 50 revealed a low level concentration of chlorpyrifos-methyl (0.251 ppm; 0.0126 mg/kg/day) in the control diet prepared from one of three lots of feed used in this study and it was suggested that the test material was present as a constituent of the feed itself. This lot (OCT07871F) of Purina Certified Rodent Chow #5002 was used in the preparation of 3 (#6, #7, and #8) of the 13 total study diets. Rats on study received an additional 0.0126 mg/kg/day (12.6% of the lowest dose tested) in the control and treated diets for 3 weeks (study days 36-57).

4. Statistics: From the study report, pp. 21

"Body weights and gains, organ weights, clinical chemistry data, hematology data and

urinary specific gravity data were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or non parametric analysis of variance (ANOVA), followed respectively by Dunnett's test for Wilcoxon's test with a Bonferroni correction for multiple comparisons. Statistical outliers were identified by a sequential test, but routinely excluded only from feed consumption means."

"The nominal alpha levels used and the test references were as follows:

Bartlett's test (Winer, 1971)	0.01
Parametric ANOVA (Steel & Torrie, 1960)	0.10
Nonparametric ANOVA (Hollander & Wolfe, 1973)	0.10
Dunnett's test (Winer, 1971)	0.05, 2-sided
Wilcoxon's test (Hollander & Wolfe, 1973)	0.05, 2-sided
Bonferroni correction (Miller, 1966)	0.05, 2-sided
Outlier test (Grubbs, 1969)	0.02, 2-sided"

C. METHODS

1. Observations

Animals were observed daily (including weekends) for mortality and signs of toxicity. Clinical examination was performed on each animal by a veterinarian; once during the acclimatization period (study day -14; 2 weeks prior to the initiation of the study) and thereafter, at least once weekly.

2. Body weight

Animals were weighed before study initiation (once during the 14 day acclimatization period), on the day preceding the first administration of test compound (study day -1; baseline body weight), once weekly throughout the treatment period and prior to necropsy (study days 92 for males and 93 for females and recovery day 121 for males and females)

3. Food consumption

Food (Feed) consumption, expressed as grams/rat/day, was determined weekly for each animal during the treatment period. Food conversion efficiency was not determined.

4. Ophthalmologic examination - Not performed.

5. Blood

Blood was drawn by orbital sinus puncture immediately prior to scheduled sacrifice at 13 weeks for primary group rats and at 17 weeks for recovery group rats for hematology and clinical chemistry. Animals were fasted overnight prior to bleeding and lightly anesthetized during blood collection.

a. Hematology - parameters measured on study days 92, 93 or 121.

x	Packed Cell Volume	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Platelet count (PLT)*
x	Leukocyte count (WBC)*		Blood clotting measurement's*
x	Erythrocyte count (RBC)*		(pro thrombin time)

* Required for subchronic toxicity studies based on Subdivision F Guidelines

b. Clinical Chemistry - parameters measured on study days 92, 93 or 121.

	ELECTROLYTES		OTHER
x	Calcium (CA)*	x	Albumin (ALB)*
x	Chloride (CL)*	x	Blood creatinine (CREA)*
x	Inorganic Phosphate (PHOS)*	x	Blood urea nitrogen*
x	Potassium (K)*	x	Total Cholesterol (CHOL)
x	Sodium (NA)*	x	Triglycerides (TRIG)
		x	Glucose (GLUC)*
	ENZYMES	x	Total bilirubin (TBIL)
		x	Total serum protein (TPRO)*
x	Alkaline phosphates (ALP)	x	Globulin (GLOB)
	Creatine kinase (CK)*		
	Lactate dehydrogenase (LD)*		
x	Serum alanine aminotransferase (ALAT; also ALT, SGPT)*		
x	Serum aspartate aminotransferase (ASAT; also AST, SGOT)*		

* Required for subchronic toxicity studies based on Subdivision F Guidelines.

c. Cholinesterase Activity - From the study report, pp. 19

"Plasma and red blood cell cholinesterase levels were evaluated in samples taken from all primary animals on Study Days 44 (males) and 45 (females), from all primary animals at the 13-week necropsy, and from all recovery animals at the 17-week necropsy. Brain cholinesterase was determined in half brain samples taken from all animals at the 13- or 17-week necropsies. The remaining half-brains were preserved in neutral phosphate-buffered 10% formalin."

6. Urinalysis-Although analysis of urine in a subchronic mammalian toxicity study is not required according to the Subdivision F Guidelines, urinalysis was performed prior to sacrifice at the 13 and 17 week sampling time (study day 87 for primary group rats and day 120 for recovery group rats). Measurements included specific gravity, pH, protein, sugar, ketones, bilirubin, blood and urobilinogen. Sediment from a pooled sample/sex/group was examined microscopically.

7. Functional Observational Battery- From the study report, pp. 19

"An observational battery was performed on all primary group rats on Study Days 10, 30, and 86, and on all recovery rats on Study Day 115. The rats were removed from their cages one at a time, and the handler rated evasive behavior at the time of capture. A second observer, blind to the identity of the animal, evaluated the rat for muscle tone, tremor, haircoat condition, salivation, lacrimation, urine staining, fecal staining, locomotor activity, response to touch, response to sound, and response to tail pinch. A third participant recorded the observations."

8. Sacrifice and Pathology

a. Gross and microscopic examination

All animals were fasted overnight prior to necropsy, sacrificed by decapitation and subjected to gross pathological examination on study days 92, 93 or 121. The checked (x) tissues were collected from all animals in the control and high dose groups for histological examination and, in addition, the checked (xx) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
x	Tongue	x	Aorta*	xx	Brain (cerebellum,
x	Oral tissue	xx	Heart*		forebrain, medulla
x	Salivary gland	x	Bone marrow*		oblongata, midbrain)*
x	Esophagus*	x	Lymph nodes	x	Spinal cord
x	Stomach*		(mediastinal,mesenteric)*		(3 levels)* [T]
x	Duodenum*	x	Lymph tissue	x	Pituitary*
x	Jejunum*		(mediastinal, mesenteric)	x	Eyes (optic nerve)* [‡]
x	Ileum*	x	Spleen*	x	Peripheral Nerves
x	Cecum*	x	thymus*		(sciatic, tibial)
x	Colon				
x	Rectum*		UROGENITAL		GLANDULAR
xx	Liver* [†]				
x	Pancreas*	xx	Kidney's* [†]	xx	Adrenal gland*
		x	Urinary bladder*	x	Lacrimal/Harderian
	RESPIRATORY	xx	Testes* [†]		gland [T]
		x	Epididymides	x	Mammary gland [T]
x	Trachea*	x	Prostate	x	Thryoid* [††]
x	Lung*	x	Seminal vesicle	x	Parathyroid* [††]
x	Larynx	xx	Ovaries* [†]		
x	Nasal tissue	x	Oviduct		OTHER
		x	Uterus*		
		x	Vagina	x	Bone*
		x	Cervix	x	Skeletal muscle*
				x	Skin*
					All gross lesions and Masses*

*Required for subchronic toxicity studies based on Subdivision F Guidelines.

[†]Organ weight required in subchronic studies.

[‡]Organ weight required for non-rodent studies.

[T]Required only when toxicity present or target organ.

II. RESULTS

A. OBSERVATIONS

1. Mortality: No animals died during the study or recovery periods.

2. Clinical signs: Clinical signs involving the eye were judged not to be treatment-related because these effects occurred infrequently (phthopsis in one primary group female rat and red-crusted, matted shut eyelids in one recovery control group female rat) and may have resulted from injury sustained at the time of orbital sinus sampling. Additional clinical signs noted weekly and during the three functional observational battery evaluations included decreased condition of the haircoat, urine staining and increased lacrimation in high-dose rats. (See Section G. Functional Observational Battery).

B. BODY WEIGHT

Body weights and body weight gains in male and female rats are summarized in Tables 2 and 3. Mean body weights of male rats dosed at 250 mg/kg/day were significantly decreased 3.0-10.1% throughout the13 week treatment period and remained slightly, but not significantly decreased (3.6%) by the end of the recovery period (study week 17). Similarly, female rats in the high dose group had significantly lower body weights compared to control animals (5.0-14.1%) during the treatment period. By the fourth week of the recovery period, decreases in female body weights were partially reversed (7.1%) but still statistically significant. Compared to control animals, mean body weight gains were significantly decreased in high-dose males (17.2%) and in high-dose females (26.3%) during the dosing interval (study days 0-91) but were not decreased by the end of the recovery period (study week 17).

Table 3. Body Weight and Body Weight Gain in Male Rats[a]

Dose (mg/kg/day)					
Study Day	**0**	**0.1**	**1.0**	**10.0**	**250.0**
Body Weight in grams (mean ± S.D.)[b]					
0	126.5 ± 6.6	126.7 ± 7.0	126.0 ± 6.5	124.8 ± 7.5	128.3 ± 8.0
28	240.0 ± 10.7	238.6 ± 8.9	235.8 ± 6.8	232.7 ± 10.8	**223.5* ± 12.2**
56	285.4 ± 14.1	278.2 ± 8.2	278.4 ± 6.3	272.7 ± 13.3	**260.5* ± 17.5**
91	321.0 ± 17.6	312.2 ± 6.5	313.1 ± 7.7	308.3 ± 12.5	**289.4[s] ± 22.0**
112	327.0 ± 13.0	---	---	---	315.2 ± 24.7

Body Weight Gain in grams (% difference from control)[c]					
0 - 28	113.5	111.9 (-1.4)	109.8 (-3.3)	107.9 (-4.9)	95.2 (-16.1)
28 - 56	45.4	39.6 (-12.8)	42.6 (-6.2)	40.0 (-11.9)	37.0 (-18.5)
56 - 91	35.6	34.0 (-4.5)	34.7 (-2.5)	35.6 (0)	28.9 (-18.2)
91 -112	6.0	---	---	---	25.8 (+330)
0 - 91	194.5	185.5 (-4.6)	187.1 (-3.8)	183.5 (-5.7)	**161.1[S] (-17.2)**

[a]The number of animals for the control and high-dose groups were 20 rats/sex for the first thirteen weeks and 10 rats/sex for all other weighings.
[b]Means and standard deviations were obtained from the study report, Table 6, pp. 42-44.
[c]Percent (%) difference from control body weight gain values for all treatment intervals were calculated by the reviewer using data provided in the study report, Table 6, pp. 42-44. Mean body weight gain was either extracted from the study report (dosing intervals; days 0-91 and 0-112), Table 6, pp. 42-46 or calculated by the reviewer for all other treatment intervals.
*Statistically different from control by Dunnett's test, $p< 0.05$, two-sided.
[S]Statistically different from control by Wilcoxin's test, $p< 0.05$, two-sided.

Table 4. Body Weight and Body Weight Gain in Female Rats[a]					
	Dose (mg/kg/day)				
Study Day (s)	**0**	**0.1**	**1.0**	**10.0**	**250.0**
Body Weight (grams)[b]					
0(-1)	97.6 ± 5.7	96.1 ± 5.6	96.7 ± 3.5	97.3 ± 4.0	96.0 ± 4.1
28	152.9 ± 6.1	150.6 ± 7.7	153.4 ± 6.6	152.0 ± 6.7	**141.3* ± 6.0**
56	177.9 ± 8.5	174.7 ± 9.6	175.2 ± 7.2	173.3 ± 8.3	**155.5* ± 8.1**
91	193.0 ± 7.6	190.6 ± 8.4	190.7 ± 6.8	186.8 ± 8.0	**166.3* ± 9.9**
112	193.7 ± 5.8	---	---	---	**179.9* ± 10.8**

Body Weight Gain in grams (% difference from control)[c]					
0(-1) - 28	55.3	54.5 (-1.4)	56.7 (+2.5)	54.7 (-1.1)	45.3 (-18.1)
28 - 56	25.0	24.1 (-3.6)	21.8 (-12.8)	21.3 (-14.8)	14.2 (-43.2)
56 - 91	15.1	15.9 (+5.3)	15.5 (+2.7)	13.5 (-10.6)	10.8 (-28.5)
91 -112	0.7	---	---	---	13.6 (+1842.9)
0 - 91	95.4	94.5 (-0.9)	94.0 (-1.5)	89.5 (-6.2)	**70.3* (-26.3)**

[a]The number of animals for the control and high-dose groups were 20 rats/sex for the first thirteen weeks and 10 rats/sex for all other weighings.
[b]Means and standard deviations were obtained from the study report, Table 7, pp. 45-47.
[c]Percent (%) difference from control body weight gain values for all treatment intervals were calculated by the reviewer using data provided in the study report,Table 7, pp. 45-47. Mean body weight gain was either extracted from the study report (dosing intervals; days 0-91 and 0-112),Table 7, pp. 45-47 or calculated by the reviewer for all other treatment intervals.
*Statistically different from control by Dunnett's test, $p< 0.05$, two-sided.

C. FOOD CONSUMPTION

Food consumption was not analyzed for differences of statistical significance. However, high-dose females appeared to consume less food (3.3-13%) compared to control animals throughout the13 week treatment period. This decrease was almost completely reversed (-1.5%) by the end of the recovery period (Table 4). Food conversion efficiency was not determined .

Table 5. Food Consumption in grams/animal/day [a] (% difference from control)[b]					
Dose (mg/kg/day)					
Treatment	**0**	**0.1**	**1.0**	**10.0**	**250.0**
Males (mean ± S.D.)[c]					
Week 1	14.5 ± 0.7	14.7 ± 0.8 (+1.4)	14.5 ± 0.9 (0)	14.2 ± 1.1 (-2.1)	14.7 ± 1.0 (+1.4)
Week 4	17.4 ± 0.8	17.3 ± 0.8 (-0.5)	17.2 ± 0.6 (-1.2)	17.1 ± 0.7 (-1.7)	16.6 ± 0.8 (-4.6)
Week 8	18.1 ± 0.9	17.8 ± 0.8 (-1.7)	17.9 ± 0.7 (-1.1)	17.5 ± 1.1 (-3.3)	16.8 ± 1.2 (-7.1)
Week 13	18.3 ± 1.0	17.8 ± 0.8 (-2.7)	17.8 ± 0.8 (-2.7)	17.6 ± 0.9 (-3.8)	18.4 ± 1.1 (+0.6)
Week 17	18.0 ± 0.7	---	---	---	18.7 ± 1.4 (+3.9)

Females (mean ± S.D.)[c]					
Week 1	12.1 ± 0.6	11.7 ± 0.7 (-3.3)	12.0 ± 0.6 (-0.8)	12.0 ± 0.5 (-0.8)	11.7 ± 0.7 (-3.3)
Week 4	13.7 ± 0.6	13.4 ± 1.0 (-2.2)	13.7 ± 1.3 (0)	13.1 ± 0.9 (-4.4)	12.6 ± 0.9 (-8.0)
Week 8	13.8 ± 0.8	13.7 ± 1.0 (-0.7)	13.3 ± 1.4 (-3.6)	13.2 ± 1.1 (-4.3)	12.0 ± 0.8 (-13.0)
Week 13	13.6 ± 0.5	12.9 ± 0.4 (-5.2)	13.2 ± 0.7 (-2.9)	12.5 ± 0.4 (-8.1)	12.4 ± 0.9 (-8.8)
Week 17	13.4 ± 1.0	---	---	---	13.2 ± 0.9 (-1.5)

[a] Means and standard deviations were obtained from the study report, Tables 4 and 5, pp. 38-41.
[b]The % difference from control food consumption values was calculated by the reviewer using data provided in the study report, Tables 4 and 5, pp. 38-41.
[c]The number of animals for the control and high-dose groups were 20 rats/sex for the first thirteen weeks and 10 rats/sex for all other weighings.

D. OPHTHALMOLOGIC EXAMINATION

Not performed.

E. BLOOD WORK

1. Hematology

At 250 mg/kg/day, red blood cells, hemoglobin and packed cell volume values were significantly decreased by 11.0, 10.6 and 8.1%, respectively, in male rats and by 14.1, 12.1 and 9.9%, respectively, in female rats and remained slightly (3.0-3.5% in male rats and 0.73-2.7% in female rats), but significantly decreased at the end of the recovery. No significant changes in these hematology parameters were observed in rats receiving dietary doses of ≤10 mg/kg/day chlorpyrifos-methyl.

Statistically significant increases of 23.3, 14.9, 8.0 and 22.2% above control platelet count values occurred at dose levels of 0.1, 1.0, 10 and 250.0 mg/kg/day, respectively, in female rats. However, the increase in the number of platelets was not dose-related and by recovery week 17, there was evidence of reversal in the increases observed in high-dose females (2.9% increase above controls). Platelet counts in high-dose males were significantly increased by 21.9% and 13.8% at 13 and 17 weeks, respectively.

Occasional changes observed in both the white blood cell counts and the WBC differential counts were not statistically significant and did not show a dose relationship.

2. Clinical Chemistry

Group mean alkaline phosphatase, alanine transaminase and aspartate transaminase levels in high dose animals at 13 weeks were decreased by 21, 20.3 and 10.2% (not significant) in males and by 22.8, 31.9 and 31.6% in females. At recovery week 17, these enzyme levels remained decreased by 11.5, 16.9 and 2.6% (not significant) in males and by 9.2, 24.3 and 28% in females. Cholesterol levels were significantly increased at 13 weeks in high-dose males (24.7%) and females (57.5 %) but were comparable to control values at 17 weeks. Creatinine levels were decreased by 23.4% in high-dose females at 13 weeks and remained decreased (25.6%) at 17 weeks. Although urea nitrogen values were significantly decreased in male rats at dose levels of 1 and 10 mg/kg/day, a statistically significant increase (10.6%) occurred at 250 mg/kg/day. Other statistically significant changes were seen in treated groups compared to controls, but the changes appeared to be random events and were not dose-related.

3. Cholinesterase Activity

Cholinesterase activity in primary and recovery group rats is presented in Tables 5 and 6. Measurements of plasma cholinesterase activity at 6 weeks showed significant dose-related decreases; cholinesterase activity was inhibited by 12.5, 26.3 and 48.7% (males) and by 34.1, 71.5 and 87.3% (females) at 1, 10 and 250 mg/kg/day, respectively. Following the 13 week dosing interval, plasma cholinesterase activity was also significantly decreased in a dose-dependent manner in males (7.3, 20.4, 33.8 and 53.7% at dose levels of 0.1, 1, 10 and 250 mg/kg/day, respectively) and in females (33.8, 72.9 and 86.6% at dose levels of 1, 10 and 250 mg/kg/day, respectively).

Dose-dependent decreases were observed in red blood cell cholinesterase activity (6.2 [ns], 15.6 [ns], 25.5 and 30.1%) of males at 6 weeks and in brain cholinesterase activity (2.3 [ns], 3.9 [ns], 17.0 and 62.6%) of males at 13 weeks at dose levels of 0.1, 1, 10 and 250 mg/kg/day, respectively. In female rats, red blood cell cholinesterase activity was decreased (7.6 [ns], 11.7 [ns], 31.5 and 25.4% at dose levels of 0.1, 1, 10 and 250 mg/kg/day, respectively) at 6 weeks and remained relatively unchanged (27.7% at a dose level of 250 mg/kg/day) at 13 weeks. Inhibition of brain cholinesterase activity in females was observed at 0.1 (90.0%, ns), 1 (1.2%, ns), 10 (14.5%) and 250 mg/kg/day (58.9%) by 13 weeks.

The 17-week recovery interval animals showed either a partial or almost complete reversal of cholinesterase inhibition at 250 mg/kg/day; plasma cholinesterase inhibition was 7% (males) and 1.9% (females, ns); red blood cell cholinesterase inhibition was 11.7% (males) and 17.6% (females); and brain cholinesterase inhibition was 19.3% (males) and 17.3% (females).

Table 6. Cholinesterase Activity in Male Rats [a] (% difference from control) [b]

Dose (mg/kg/day)	Plasma (units/mL)			RBC (units/mL)			Brain (units/g)	
	6 weeks	13 weeks	17 weeks	6 weeks	13 weeks	17 weeks	13 weeks	17 weeks
0	0.649 ± 0.041	0.631± 0.033	0.590 ± 0.050	2.176 ± 0.230	2.248 ± 0.322	2.176 ± 0.284	10.554 ± 0.776	10.382 ± 0.547
0.1	0.649 ± 0.046 (0)	**0.585* ± 0.022** (-7.3)	---	2.042 ± 0.436 (-6.2)	2.522 ± 0.340 (+12.2)	---	10.308 ± 0.600 (-2.3)	---
1.0	**0.568* ± 0.023** (-12.5)	**0.502* ± 0.025** (-20.4)	---	1.836 ± 0.322 (-15.6)	2.072 ± 0.420 (-7.8)	---	10.145 ± 0.564 (-3.9)	---
10.0	**0.478* ± 0.021** (-26.3)	**0.418* ± 0.019** (-33.8)	---	**1.622* ± 0.373** (-25.5)	1.907 ± 0.448 (-15.2)	---	**8.765* ± 0.717** (-17.0)	---
250.0	**0.333* ± 0.017** (-48.7)	**0.292* ± 0.020** (-53.7)	**0.549* ± 0.049** (-7.0)	**1.522* ± 0.340** (-30.0)	1.874 ± 0.594 (-16.6)	**1.922* ± 0.213** (-11.7)	**3.952* ± 0.721** (-62.6)	**8.378* ± 0.435** (-19.3)

[a]Means and standard deviations were extracted from Appendix Tables 16, 17 and 18, pp. 146-150 of the study report.

[b]Percent (%) difference from control cholinesterase values were calculated by the reviewer using data provided in the study report, Appendix Tables 16, 17 and 18, pp. 146-15.

*Statistically different from control by Dunnett's test, $p < 0.05$, two-sided.

Table 7. Cholinesterase Activity in Female Rats [a] (% difference from control) [b]								
Dose (mg/kg/day)	Plasma (units/mL)			RBC (units/mL)			Brain (units/g)	
	6 weeks	13 weeks	17 weeks	6 weeks	13 weeks	17 weeks	13 weeks	17 weeks
0	2.818 ± 0.310	2.662± 0.552	3.193 ± 0.268	2.426 ± 0.291	1.518 ± 0.282	2.044 ± 0.228	10.345 ± 0.520	10.551 ± 0.596
0.1	2.931 ± 0.278 (+4.0)	3.012 ± 0.404 (+13.1)	---	2.242 ± 0.341 (-7.6)	1.502 ± 0.338 (-1.05)	---	10.317 ± 0.632 (-90.0)	---
1.0	**1.857[$] ± 0.228** (-34.1)	**1.762[$] ± 0.086** (-33.8)	---	2.142 ± 0.615 (-11.7)	1.584 ± 0.216 (+4.3)	---	10.189 ± 0.395 (-1.2)	---
10.0	**0.803[$] ± 0.132** (-71.5)	**0.721[$] ± 0.066** (-72.9)	---	**1.662* ± 0.447** (-31.5)	1.362 ± 0.281 (-10.3)	---	**8.843* ± 0.666** (-14.5)	---
250.0	**0.359[$]± 0.023** (-87.3)	**0.358[$] ± 0.054** (-86.6)	3.132 ± 0.261 (-1.9)	**1.810* ± 0.322** (-25.4)	**1.098* ± 0.361** (-27.7)	**1.684* ± 0.505** (-17.6)	**4.255* ± 0.529** (-58.9)	**8.728* ± 0.535** (-17.3)

[a]Means and standard deviations were extracted from Appendix Tables 16, 17 and 18, pp. 146-150 of the study report.
[b]Percent (%) difference from control cholinesterase values were calculated by the reviewer using data provided in the study report, Appendix Tables 16, 17 and 18, pp. 146-15.
*Statistically different from control by Dunnett's test, $p< 0.05$, two-sided.
[$]Statistically different from control by Wilcoxin's test, $p< 0.05$, two-sided.

F. URINALYSIS

Urinalysis parameter values were comparable to control values at all doses tested, with the exception of specific gravity. A statistically significant decrease in the specific gravity of high-dose males at 13 weeks did not persist in the recovery males at 17 weeks and was absent in primary and recovery group female rats. The percent decrease (2.6%) in the specific gravity of the urine measured only in high-dose male rats is considered negligible.

G . FUNCTIONAL OBSERVATIONAL BATTERY

At each of the 3 observational battery evaluations (study days 10, 30 and 86), urine staining, decreased condition of the haircoat and increased lacrimation occurred almost entirely in the 250 mg/kg/day chlorpyrifos-methyl dose group and these effects were observed most often in female rats compared to male rats. At the end of the 4 week recovery period, only urine staining was still present in 9/10 high-dose females and 1/10 high-dose males. Urine staining was also present in some control, low-dose and mid-dose female rats at the 30 and/or 86 day evaluations. The presence of red pigment in the eye area occurred sporadically in primary group and recovery group female rats and there was no correlation with dosing.

H. SACRIFICE AND PATHOLOGY

1. Organ weight

Selected 13-week and 17-week organ weights are summarized in Tables 7 and 8. The absolute and relative group mean adrenal weights were significantly increased at 13 weeks by 58.0% and 81.6%, respectively, in high-dose males and by 64% and 90%, respectively, in high-dose females. Adrenal weights, both absolute and relative, were also significantly increased in females by 40.0% and 45.8%, respectively, at 10 mg/kg/day, whereas, absolute adrenal gland weights in males was significantly increased by 10.0%. The absolute and relative liver weights of high-dose animals were significantly increased by 22.5% and 39.0%, respectively, in males and 20.8% and 40.2%, respectively, in females. Relative kidney, brain, heart and testes organ weights in high-dose males and relative kidney, brain, and heart organ weights in high-dose females were significantly increased in primary group rats.

At 17 weeks, recovery group absolute organ weights were comparable to control group weights at all doses tested. However, high-dose male relative adrenal, liver and kidney organ weights and high-dose female relative adrenal, liver, kidney and brain weights remained significantly increased over control group weights.

Table 8. Absolute and Relative (to Body weight) Organ Weights in Male Rats [a] (% difference from control) [b]					
Organ	Dose (mg/kg/day)				
	0	0.1	1.0	10.0	250.0
13 Week Sacrifice					
Terminal body weight	304.0±20.6	290.5±7.12	293.0±7.7	288.4±12.6	**267.6[S]±20.8** (-12.0)
Adrenal Glands absolute weight	0.050±0.004	**0.042*±0.005** (-16.0)	**0.043*±0.005** (-14.0)	0.055±0.004 (+10.0)	**0.079*±0.007** (+58.0)
Adrenal Glands relative weight	0.0163±0.00145	0.0146±0.0016 (-10.4)	0.0148±0.0017 (-9.2)	**0.0190*±0.0016** (+16.6)	**0.0296*±0.0034** (+81.6)
Liver absolute weight	8.360±0.571	7.770±0.280 (-7.1)	7.958±0.368 (-4.8)	7.721±0.564 (-7.6)	**10.237[S]±1.070** (+22.5)
Liver relative weight	2.751±0.0.102	2.676±0.089 (-2.7)	2.717±0.117 (-1.2)	2.675±0.090 (-2.8)	**3.823*±0.229** (+39.0)
Kidneys relative weight	0.681±0.043	0.713±0.029 (+4.7)	0.692±0.020 (+1.6)	0.697±0.024 (+2.3)	**0.776*±0.036** (+14.0)
Brain relative weight	0.631±0.037	0.656±0.015 (+4.0)	0.657±0.021 (+4.1)	0.658±0.020 (+4.3)	**0.721[S]±0.048** (+14.3)
Heart absolute weight	0.903±0.029	0.871±0.048 (-3.5)	0.873±0.043 (-3.3)	0.865±0.061 (-4.2)	**0.802*±0.050** (-11.2)
Testes relative weight	1.009±0.020	**1.051[S]±0.030** (+4.1)	1.054±0.049 (+4.5)	**1.066[S]±0.049** (+5.7)	**1.132[S]±0.079** (+12.2)
17 Week Sacrifice					
Terminal body weight	311.4±12.3	---	---	---	298.5±22.2
Adrenal Glands relative weight	0.0145±0.0011	---	---	---	**0.163*±0.0019** (+12.4)
Liver relative weight	2.606±0.082	---	---	---	**2.833*±0.093** (+8.7)
Kidneys relative weight	0.660±0.019	---	---	---	**0.829[S]±0.448** (+25.6)

[a]Means and standard deviations were extracted from Tables 19-22, pp. 62-65 of the study report.
[b]Percent (%) difference from control absolute and relative organ weight values were calculated by the reviewer using data provided in the study report, Tables 19-22, pp. 62-65.

*Statistically different from control by Dunnett's test, p< 0.05, two-sided.
[S]Statistically different from control by Wilcoxin's test, p< 0.05, two-sided.

Table 9. Absolute and Relative (to Body weight) Organ Weights in Female Rats [a] (% difference from control) [b]

Organ	Dose (mg/kg/day)				
	0	0.1	1.0	10.0	250.0
13 Week Sacrifice					
Terminal body weight	180.3±8.3	177.4±8.6	178.0±6.9	174.1±7.6	**155.4*±7.9** (-13.8)
Adrenal Glands absolute weight	0.050±0.004	0.051±0.007 (+2.0)	0.054±0.006 (+8.0)	**0.070*±0.006** (+40.0)	**0.082*±0.007** (+64.0)
Adrenal Glands relative weight	0.0277±0.0022	0.0285±0.0038 (+2.9)	0.0304±0.0028 (+9.7)	**0.0404*±0.0034** (+45.8)	**0.0526*±0.0034** (+89.9)
Liver absolute weight	4.845±0.275	4.874±0.258 (+0.6)	4.766±0.247 (-1.6)	4.764±0.353 (-1.7)	**5.854*±0.298** (+20.8)
Liver relative weight	2.687±0.102	2.749±0.101 (+2.3)	2.676±0.077 (-0.4)	2.735±0.119 (+1.8)	**3.768[S]±0.094** (+40.2)
Kidneys relative weight	0.743±0.027	0.754±0.400 (+1.5)	0.764±0.031 (+2.8)	0.759±0.031 (+2.2)	**0.894*±0.030** (+20.3)
Brain relative weight	0.987±0.043	1.005±0.045 (+1.8)	0.998±0.036 (+1.1)	1.011±0.038 (+2.4)	**1.136*±0.048** (+15.1)
Heart relative weight	0.347±0.019	0.362±0.022 (+4.3)	0.350±0.010 (+0.9)	0.346±0.013 (-0.3)	**0.375*±0.017** (+8.1)
17 Week Sacrifice					
Terminal body weight	180.9±5.3	---	---	---	**168.9*±11.6** (-6.6)
Adrenal Glands relative weight	0.0279±0.0042	---	---	---	**0.0320*±0.0034** (+14.7)
Liver relative weight	2.646±0.089	---	---	---	**2.849*±0.266** (+7.7)
Kidneys relative weight	0.719±0.024	---	---	---	**0.797*±0.049** (+10.9)
Brain relative weight	0.978±0.029	---	---	---	**1.061[S]±0.083** (+8.5)

[a]Means and standard deviations were extracted from Tables 19-22, pp. 62-65 of the study report.
[b]Percent (%) difference from control absolute and relative organ weight values were calculated by the reviewer using data provided in the study report, Tables 19-22, pp. 62-65.
*Statistically different from control by Dunnett's test, $p < 0.05$, two-sided.
[s]Statistically different from control by Wilcoxin's test, $p < 0.05$, two-sided.

2. Gross pathology

The gross lesion attributed to chlorpyrifos-methyl administration occurred at the dose level of 250 mg/kg/day and consisted of perineal staining in male (10/10) and female (9/10) primary group rats, but was observed mostly in females (1/10 males, 9/10 females) at the 17 week necropsy.

3. Microscopic pathology

Selected microscopic lesions identified in male and female rats at 13 week and 17 week sacrifice are summarized in Tables 9 and 10, respectively. In primary group male and female rats fed 10 or 250 mg/kg/day treated diets, lesions identified in the adrenal glands of all animals at those doses consisted of slight to moderate hypertrophy and vacuolation of the cells of the zona fasciculata. Also noted within the zona fasciculata of female rats were scattered necrotic foci, suggesting that the pathologic process within the adrenal glands may have progressed to very slight or slight necrosis at 10 and 250 mg/kg/day, respectively. At the 17 week necropsy, recovery group male rats showed a reduction in the severity of adrenal hypertrophy and vacuolation and the adrenal hypertrophy and scattered necrotic foci were no longer present in female rats.

Microscopic examination of the liver consisted of very slight hypertrophy of centrilobular hepatocytes observed only in males at the 250 mg/kg/day dose. By the end of the recovery period, hypertrophy of centrilobular hepatocytes was absent in male rats. Other microscopic lesions (including kidney mineralization, very slight to slight degeneration/regeneration and dilated tubules and tongue mineralization) occurred at similar frequencies in control and treated groups, were observed only sporadically or were no longer present by study week 17.

Table 10. Selected microscopic lesions in primary group male and female rats at 13 week sacrifice .

Organ	Dose (mg/kg/day)				
	0	**0.1**	**1.0**	**10.0**	**250.0**
Males (n = 10)					
Adrenals, hypertrophy, slight to moderate	0	0	0	10	10
Adrenals, vacuolation, slight to moderate	0	0	0	10	10
Liver, hypertrophy, centrilobular, very slight	0	0	0	0	7
Liver, necrosis with inflammation, multifocal, very slight	1	1	1	4	0
Kidneys, Dilated tubules	4	1	2	2	7
Kidneys, Mineralization	10	10	10	10	10
Kidneys, Degeneration/Regeneration, very slight to slight	10	10	10	9	10
Females (n = 10)					
Adrenals, hypertrophy, slight to moderate	0	0	0	10	10
Adrenals, vacuolation, slight to moderate	0	0	0	10	10
Adrenals, necrosis, very slight to slight	0	0	0	4	8
Liver, necrosis with inflammation, multifocal, very slight	3	1	1	2	0
Kidneys, Mineralization	10	10	10	10	10
Kidneys, Degeneration/Regeneration, very slight	1	1	0	1	0

Data were extracted from Tables 25 and 26, pp. 68-83 of the study report.

Table 11. Selected microscopic lesions in recovery group male and female rats at 17 weeks sacrifice.

Organ	Dose (mg/kg/day)				
	0	0.1	1.0	10.0	250.0
Males (n = 10)					
Adrenals, hypertrophy, slight	0	---	---	---	10
Adrenals, vacuolation, very slight to slight	0	---	---	---	10
Kidneys, Dilated tubules	4	---	---	---	5
Kidneys, Mineralization	10	---	---	---	10
Kidneys, Degeneration/Regeneration, very slight	10	---	---	---	10
Tongue, mineralization	9	---	---	---	10
Females (n = 10)					
Adrenals, vacuolation, very slight	0	---	---	---	10
Liver, necrosis with inflammation, multifocal, very slight	2	---	---	---	0
Kidneys, Mineralization	10	---	---	---	9
Kidneys, Degeneration/Regeneration, very slight	0	---	---	---	2

Data were extracted from Tables 25 and 26, pp. 68-83 of the study report.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The study author concluded that the subchronic dietary no-observed-adverse-effect-level (NOAEL) for chlorpyrifos-methyl insecticide treatment in rats was 0.1 mg/kg/day. This conclusion was based on plasma cholinesterase inhibition at doses ≥1.0 mg/kg/day.

B. REVIEWER'S DISCUSSION

No mortalities occurred during the study and recovery periods in either sex.

The general health status of most rats was considered unremarkable with the exception of clinical signs observed in two animals. These clinical signs, involving the eye, were judged not to be treatment-related because this effect occurred infrequently (phthopsis in one primary group female rat and red-crusted, matted shut eyelids in one recovery control group female rat) and may have resulted from injury sustained at the time of orbital sinus sampling.

The clinical signs attributed to chlorpyrifos-methyl administration which were noted weekly and during the three functional observational battery (FOB) evaluations included decreased condition of the haircoat, urine staining and increased lacrimation in both sexes. These effects occurred almost entirely in the 250 mg/kg/day chlorpyrifos-methyl dose group and were observed most often in female rats compared to male rats. At the end of the 4 week recovery period, only urine staining was still present in 9/10 high-dose females and 1/10 high-dose males.

Throughout the 13-week treatment interval, mean body weights were significantly decreased following chlorpyrifos-methyl treatment in male and female rats at a dose level of 250 mg/kg/day. Mean body weight gains were also significantly decreased in high-dose male and female rats during the dosing interval (study days 0-91). By the end of the recovery period (study week 17), the decreases in mean body weights were less pronounced and the mean body weight gains were comparable to controls. Female rats fed the 250 mg/kg/day treated diet consumed less food throughout most of the13 week treatment period, however, this decrease was almost completely reversed by study week 17. Decreases in body weight, body weight gain and food consumption are considered a treatment-related effect only at the highest dose tested.

Decreased levels in red blood cells, hemoglobin and hematocrit observed in this 90-day subchronic toxicity study are not considered a treatment-related adverse effect. The degree of changes in these parameters were small (8.1-14.1%) and there was no evidence of a dose-response (decreases occurred only at the highest dose tested, 250 mg/kg/day). In addition, there were no changes indicative of anemia at 250 mg/kg/day: values (calculated by the reviewer) for mean corpuscular volume (MCV) in males (47.6 for controls, 49.2 for the 250 mg/kg/day group) and in females (49.7 for controls, 52.0 for the 250 mg/kg/day group) and mean corpuscular hemoglobin concentration (MCHC) in males (35.5 for controls, 34.6 for the 250 mg/kg/day group) and in females (35.6 for controls, 34.9 for the 250 mg/kg/day group) were well within normal range. Furthermore, slightly decreased red blood cell, hemoglobin and hematocrit (packed cell volume) values observed in rats exposed to chlorpyrifos-methyl in the two-year combined chronic toxicity/carcinogenicity study (MRID 42269001) at 1 and 50 mg/kg/day were considered unremarkable because the changes were small (<10%) and only detected at the 6 and 12 month sampling intervals.

Statistically significant increases above control platelet count values occurred in high-dose females at dose levels of 0.1 mg/kg/day and above. By study week 17, there was evidence of a reversal in the increased platelet counts in all doses tested. Platelet counts in high-dose males were significantly increased by 21.9% and 13.8% at 13 and 17 weeks, respectively. Although the increases in platelet counts at 250 mg/kg/day are considered a high dose treatment-related effect in both sexes, these changes may not be toxicologically significant.

At 250 mg/kg/day, group mean alkaline phosphatase, alanine transaminase and aspartate transaminase values were decreased in male and female rats; by recovery week 17, these enzyme activity levels remained decreased, but to a lesser extent than the values observed at 13 weeks. At the completion of the dosing period, cholesterol levels were significantly increased in high-dose males and females but were comparable to control values at 17 weeks. Creatinine levels were decreased in high-dose females only at 13 weeks and remained decreased by the end of the recovery period. Urea nitrogen was decreased in male rats at dose levels of 0.1 mg/kg/day and above, however, the decreases were not dose-related and a slight but significant increase occurred at 250 mg/kg/day. Although the changes observed in hepatic enzyme levels, cholesterol, creatinine, urea nitrogen achieved statistical significance, the increases/decreases in these parameters are not indicative of any disease process and therefore, are of no toxicological significance.

Measurements of plasma cholinesterase activity at 6 weeks showed significant dose-related decreases in male and female rats at 1 mg/kg/day and above. Following the 13 week dosing interval, plasma cholinesterase activity was also significantly decreased in a dose-dependent manner (males: at dose levels of 0.1 mg/kg/day and above) and (females: (at dose levels of 1 mg/kg/day and above). At 10 and 250 mg/kg/day, significant decreases were observed in the 6 week red blood cell cholinesterase and the13 week brain cholinesterase activity levels of male rats. Similarly, the 6 week red blood cell cholinesterase activity level was significantly decreased at 10 and 250 mg/kg/day, but remained significantly inhibited and relatively unchanged only at 13 weeks in the high-dose group female rats. Brain cholinesterase activity at 13 weeks was significantly inhibited in the 10 and 250 mg/kg/day group female rats. All rats showed either partial or nearly complete reversal of plasma, red blood cell and brain cholinesterase inhibition by the end of the recovery period (study week 17). The inhibition of cholinesterase activity is considered treatment-related.

The absolute and relative group mean adrenal weights were significantly increased at 13 weeks in high-dose male and female rats. At 10 mg/kg/day, absolute and relative adrenal weights were significantly increased in females, whereas, only absolute adrenal gland weights in males were increased. Similarly, absolute and relative livers weights of high-dose animals were found to be increased in both sexes. At 17 weeks, recovery group absolute organ weights were comparable to control group weights at all doses tested. Although significant increases in relative organ weights occurred in primary group males (kidney, brain, heart and testes) and females (kidney, brain, and heart) at 13 weeks and persisted to 17 weeks in recovery group males (adrenal, liver, kidney) and females (adrenal, liver, kidney and brain), the increases in these relative organ weights alone are not attributable to a treatment-related effect, but occurred concurrently with decreased body weight.

In all primary group rats fed chlorpyrifos-methyl at dose levels of 10 mg/kg/day and above, treatment-related lesions identified in the adrenal glands consisted of hypertrophy and or vacuolation of the cells of the zona fasciculata. Also noted within the zona fasciculata were scattered necrotic foci, suggesting that the pathologic process within the adrenal glands may have progressed to necrosis in mid- and high-dose female rats. Microscopic examination of the liver revealed hypertrophy of centrilobular hepatocytes that was observed only in male rats at 250 mg/kg/day which is considered treatment-related only at the highest dose tested.

Analyses of test material concentration on study day 50 revealed a low level concentration of chlorpyrifos-methyl (0.251 ppm; 0.0126 mg/kg/day) in the control feed prepared from one of three lots of feed used in this study and it was suggested by the study authors that the test material was present as a constituent of the feed itself. This lot (OCT07871F) of Purina Certified Rodent Chow #5002 was used in the preparation of 3 (#6, #7, and #8) of the 13 total diets. Hence, rats on study received an additional 0.0126 mg/kg/day (12.6% of the lowest dose tested) in the control and treated diets for 3 weeks (study days 36-57).

The study author asserts that the "overall impact upon the study of this small quantity of chlorpyrfos-methyl was judged to be negligible". The reviewer concurs with the study authors that the presence of approximately 12.6% of the lowest test dose in the control diet for 3 (weeks 6, 7 and 8) of the 13 week exposure period did not result in a significant impact on the study. The earliest indicator and the most sensitive parameter for the presence of chlorpyrifos-methyl in this study is inhibition of plasma cholinesterase activity. In particular, female rats appear to be the most sensitive sex; plasma cholinesterase activity at 6 weeks was inhibited by 34, 72 and 87% in female rats compared to 12, 26 and 49% in male rats at dose levels of 1, 10 and 250 mg/kg /day, respectively (Table 5, pages 16-17 of this study review). When there is no contamination in the diets at week 13, the data indicates that at dose levels of 1, 10 and 250 mg/kg/day, females remain more sensitive than males to the inhibitory effects of chlorpyrifos-methyl These results are supported by the combined chronic toxicity/carcinogenicity study (MRID 42269001) in which rats were exposed to chlorpyrifos-methyl for up to 24 months. Plasma cholinesterase activity was inhibited early in the study (6 months) and to the greatest extent throughout the study interval (24 months) in female rats.

In addition, the female group dosed at 0.1 mg/kg/day showed plasma cholinesterase activity 4% greater than female control group at week 6 when the contaminated diets were used. The plasma cholinesterase levels were not significantly different in the 0.1 mg/kg/day treated male rats compared to the concurrent control male rats at this sampling time. Since a dose level of 0.1 mg/kg/day (containing an additional contaminating dose of 0.0126 mg/kg/day chlorpyrifos-methyl) in both sexes is not causing inhibition, no inhibition of plasma cholinesterase activity would be anticipated and none was observed in the control group animals that received 0.0126 mg/kg/day chlorpyrifos-methyl.

Furthermore, the toxicities noted in the adrenal glands of treated rats were also identified in the combined chronic toxicity/carcinogenicity study in rats. The adrenal cortex of male and female rats in both studies (MRID 42269001 and 44906902) showed diffuse

vacuolation consistent with lipid accumulation in the zona fasciculata and a statistically significant increase in absolute and relative adrenal weights.

It is the opinion of the reviewer that a contaminating dose of 0.0126 mg/kg/day (0.251 ppm) chlorpyrifos-methyl in the control diet prepared from one of three lots of feed used in this study did not compromise interpretation of the study results. Therefore, this study is classified **Acceptable-Guideline**.

For females, the LOAEL for plasma cholinesterase inhibition was 1.0 mg/kg/day and the NOAEL was 0.1 mg/kg/day . For males, the LOAEL for plasma cholinesterase inhibition was 0.1 mg/kg/day and the NOAEL was not established.

The LOAEL for red blood cell and brain cholinesterase inhibition was 10 mg/kg/day and the NOAEL was 1 mg/kg/day.

The LOAEL for systemic toxicity was 10 mg/kg/day based upon histopathology (hypertrophy, vacuolation and necrosis) of the adrenal glands and the NOAEL was 1 mg/kg/day.

C. STUDY DEFICIENCIES It is the opinion of the reviewer that the absence of pretest cholinesterase measurements is a minor deficiency that does not compromise the interpretation of the data presented in the study. The major deficiency of this study is the presence of chlorpyrifos-methyl as a contaminant in the control diets for three of the total thirteen study weeks. However, the study is not invalidated because there was no evidence that chlorpyrifos-methyl contamination in the feed (control diet) had any impact on the results of the study.

D. STUDY CLASSIFICATION

This study is classified **Acceptable-Guideline** and meets the guideline requirements for a subchronic toxicity study (82-1a) in rats.

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Chemical:	**Chlorpyrifos-methyl (ANSI)**
PC Code:	**059102**
HED File Code	**13000 Tox Reviews**
Memo Date:	**04/06/2000**
File ID:	**TX014122**
Accession Number:	**412-01-0121**

HED Records Reference Center
02/12/2001